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# Prevalence of Long-Term BK and JC Excretion in HIV-Infected Adults and Lack of Correlation With Serological Markers

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The natural history of polyomavirus infection, and sensitivity of diagnostic assays remain unclear. A stratified group of 94 human immunodeficiency virus (HIV)-infected patients was studied for both virological and serological markers of active infection with both JC virus and BK virus. JC DNA was detected in the urine of 18 of 81 (22%) patients and BK DNA in 30 (37%) patients. Whilst patients with a low $CD_4$ cell count ($P$ = .009), $CD_4/CD_8$ ratio ($P$ = .031) and β2M concentration ($P$ = .042) were significantly more likely to be excreting BK, JC excretion did not correlate with any of the immunological markers measured. Furthermore, when all the immunological factors were taken into account, there was no association between either BK or JC excretion and age of the patient ($P$ = .149 for BK, $P$ = 0.891 for JC). BK IgM antibody was detected in only 3 of 30 (10%) BK excretors. JC IgM was detected in 5 of 18 (27.7%) JC excretors but also in 11 of 63 (17.5%) patients without demonstrable JC excretion. Therefore IgM was a very poor indicator of viruria. One year follow-up on a subset of patients showed that both DNA detection in urine and IgM antibody remain stable over many months despite falling $CD_4$ cell counts, and would indicate that events leading to enhanced viral production probably occur early after HIV infection. Replication of JC virus in the brain leading to the onset of progressive multifocal leukoencephalopathy (PML) could not be predicted using any of the markers studied. ***J. Med. Virol. 59:474–479, 1999.*** 

**KEY WORDS:** Persistent polyomavirus infection; immunocompromised patients; serology

## INTRODUCTION

The polyomaviruses BK and JC are widespread in the human population, with adult seroprevalence rates of 75% and 55%, respectively, in the UK [Gardner, 1973; Gardner and Knowles, 1995]. Both viruses persist in the kidney and perhaps in other sites such as lymphocytes and brain, following primary infection, and can be detected by a variety of methods in the urine of many immunocompromised patients [Arthur and Shah, 1989]. More recently, results using a DNA hybridisation technique have shown that JC DNA can also be detected in the urine of many immunocompetent individuals [Kitamura et al., 1990].

JC virus has been established as the causative agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease that occurs almost always in patients whose immunity has been lowered by disease or treatment [Padgett and Walker, 1983]. Until two decades ago, PML was a rare condition, but the incidence rose subsequently due to the occurrence of the disease in up to 6% of human immunodeficiency virus (HIV)-infected patients, and indeed, PML may be the AIDS-defining illness [Snider et al., 1983; Karahalios et al., 1992; Pillay et al., 1993; Jougla et al., 1996]. The late onset of PML often during the sixth decade of life in patients immunocompromised by factors other than HIV infection [Padgett and Walker, 1983] suggested that the disease was associated with JC virus reactivation under conditions of immune suppression rather than with a primary infection. However, the factors determining the few JC-seropositive individuals who will develop the disease are unknown. BK virus has been associated with haemorrhagic cystitis in an HIV-infected patient [Gluck et al., 1994], but its wider clinical significance in this group of patients is unknown. At the molecular level it has been shown that both JC and BK can stimulate the expression of HIV [Gendelman et

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al., 1986] and conversely that the HIV tat protein can activate the JC late promoter in dually-infected glial cells [Tada et al., 1990].

To study the pattern of active BK and JC infection in HIV-infected patients, a stratified group of patients was investigated both serologically and by polymerase chain reaction (PCR), with follow-up of a selected subgroup at 1 year. Attempts were made to correlate the serological markers with virus excretion, and prognostic indicators of subsequent PML were sought.

## MATERIALS AND METHODS

### Study Group

Ninety-four HIV-infected patients, aged 20–64 years, attending the Royal Free Hospital between December 1991 and January 1992 were studied. Eighty-six patients were male (75 homosexual, 5 intravenous drug users, 1 homosexual drug user, 3 African heterosexual, 2 heterosexual) and eight female (1 intravenous drug user, 5 African heterosexual, 2 heterosexual). Fourteen patients had a $CD_4$ cell count of > 500/mm$^3$ when entering the study, 29 were between 200 and 500/mm$^3$, 25 between 50 and 200/mm$^3$, and 25 < 50/mm$^3$; in one patient the $CD_4$ cell count was unknown.

### Specimens Collected

A serum and urine specimen was collected from each patient, and stored at −30°C and −70°C, respectively, before testing. Follow-up specimens were collected from 19 patients 11–14 months later.

### Virus Serology

BK virus and JC virus haemagglutination-inhibition (HI) tests were carried out as described previously [Knowles et al., 1995]. IgM antibody was measured in an M-antibody capture radioimmunoassay (MACRIA) as described previously for BK [de Silva et al., 1995] and JC [Knowles et al., 1995]. Sera were diluted 1 in 100 in diluent (phosphate-buffered saline with 0.1% Tween 20 and 10% foetal calf serum [FCS]) and heat-inactivated at 56°C for 1 hr before testing in each MACRIA.

### PCR

Urine specimens were prepared as follows: one millilitre of urine was heated at 95°C for 10 min, allowed to cool, and spun at 11,000 rpm for 1 min. The supernatant was discarded and the pellet was resuspended in 100 μl of distilled water.

Ten microlitres of sample was used per 50-μl reaction mixture, which consisted of Gibco PCR incubation buffer, 1.5 mM $MgCl_2$, 200 μM each dNTP, and 0.625 units of Gibco Taq DNA polymerase. Nested reactions were used to amplify target sequences in the early T antigen region (common to BK and JC), and the late VP1 region of both BK and JC. The primer pairs used were described by Gibson et al. [1993] and de Silva et al. [1995], 5 pmol of each primer being used in the first round reaction and 25 pmol in the nested reaction. After initial denaturation at 94°C for 2 min 35 amplification cycles were carried out as follows: 1 min at 55°C, 1 min at 72°C and 1 min at 91°C, followed by 1 min at 55°C and a final extension at 72°C for 4 min. Two microlitres of first round product was then added to 48 μl of reaction mixture for the nested round, which consisted of 25 cycles as above. Ten μl of the final product was then run on a 2% agarose gel (SeaKem, FMC BioProducts, ME) and visualised after ethidium bromide staining. Although the PCR reaction was not quantified, an estimate of the quantity of DNA was obtained from the strength of the band obtained on the gel and whether or not the product was visible after the first round reaction. The sensitivity of the nested reactions was 1 genome equivalent for both JC and BK.

### Statistical Analysis

Single variable analysis and multivariable analysis were undertaken using Epi-Info and GLIM, respectively, on the results with regard to age, sex, $CD_4$ count, $CD_8$ count, $CD_4/CD_8$ ratio, β2M, and VP1 DNA and IgM for both BK and JC.

## RESULTS

### Seroprevalence of BK and JC in the Study Group

The initial serum samples from all 94 HIV-positive patients were tested for HI antibody to BK and JC. BK antibody was detected in 85 of the 94 patients (90.4%) at titres ranging from 1 in 5 to 1 in 2,560. Sixty-one (64.8%) of the 94 patients had antibody to JC, with titres ranging from 1 in 5 to 1 in 5,120.

### Detection of BK DNA and JC DNA in Initial Urine Samples and Correlation With Immune Status and Age

Urines from 81 patients were tested by PCR; 80 patients were seropositive for BK and 51 for JC. The common early T antigen region of the genome was detected in 38 urine samples (46.9%). BK was identified using the specific late VP1 region primers in 30 (37%) patients, one of whom was negative for the T region, and JC was identified in 18 patients (22%). Eleven patients (13.6%) excreted both BK and JC. In two positive patients the polyomavirus could not be identified, as only the early gene region was detected (common to both viruses). Thus virus excretion occurred in 30 of 80 (37.5%) patients seropositive to BK and in 18 of 51 (35%) patients seropositive to JC.

The association between BK DNA and JC DNA detection and $CD_4$ cell count is shown in Table I. Only 11 of the 81 patients had a $CD_4$ cell count of ≥ 500 cells/mm$^3$. BK DNA was detected in the urine of 9 of 40 (22.5%) patients with a $CD_4$ cell count of ≥200/mm$^3$ and in 21 of 41 (51.2%) with a $CD_4$ cell count < 200/mm$^3$ ($P$ = .009). The corresponding results for JC DNA were 9 of 40 (22.5%) and 9 of 41 (22%). ($P$ > .5). The $CD_8$ cell count was not related to either BK or JC DNA detection, whereas for BK, but not JC, the percentage positivity rose significantly as the $CD_4/CD_8$ ratio fell (Table I). There was also some evidence that BK VP1 was

TABLE I. Presence of BK DNA or JC DNA in Urine Correlated With Lymphocyte Count

| Marker | Level | No. of patients | DNA detected BK (%) | *P* | JC (%) | *P* |
|---|---|---|---|---|---|---|
| $CD_4$ | ≥200/mm$^3$ | 40 | 9 (22.5) | 0.009 | 9 (22.5) | 0.687 |
| | <200/mm$^3$ | 41 | 21 (51.2) | | 9 (22) | |
| $CD_8$ | ≥800/mm$^3$ | 44 | 16 (36.4) | 0.708 | 10 (22.7) | 0.976 |
| | <800/mm$^3$ | 37 | 14 (37.8) | | 8 (21.6) | |
| $CD_4/CD_8$ | >0.17 | 41 | 10 (24.4) | 0.031 | 7 (17.1) | 0.389 |
| | <0.17 | 40 | 20 (50) | | 11 (27.5) | |

more likely to be positive when the β2M levels were lower (< 3.0: $P = .042$).

BK DNA was detected in the urine more frequently in patients older than 35 years of age (19 of 38) than in younger patients (11 of 43) ($P = .041$), but this association was no longer significant when the immunological factors were also included in the multivariable analysis ($P = .149$). There was no correlation between JC DNA detection and the age of the patient ($P = .891$).

### IgM Antibody as a Predictor of Viral Excretion

In only 3 of the 94 patients (3.2%) was BK IgM detected, in 1 case at the low level of 1.1 units and in the other 2 cases between 5 and 10 units. All 3 patients had BK DNA viruria but so did a further 27 patients in whom BK IgM was not detected (Table II). In contrast, 16 patients (17%) had JC IgM, although in 11 of these patients the level was low (< 3 units). In only 5 of the 16 JC IgM positive patients was JC DNA detected in the urine. Conversely, JC viruria was present in 13 of 65 patients without JC IgM antibody. There was no significant association between JC IgM and JC VP1 detection ($P \geq .1$) or with any of the other markers investigated ($P \geq .1$).

### One-Year Follow-Up

Follow-up serum and urine specimens were obtained from 17 patients, all of whom were seropositive for BK and all but one of whom had antibody to JC; second urine samples were available from an additional two patients. In only one of five patients with an initial $CD_4$ count of > 200 cells/mm$^3$ did the level drop to below 200/mm$^3$ at follow-up; in five of seven patients with initial levels between 50 and 200 cells/mm$^3$, the levels dropped to 50 cells/mm$^3$ or less; and all four patients initially with a count of 10–40 cells/mm$^3$ still had a count of 10 cells/mm$^3$ at 1 year. The results of virological studies are shown in Table III. None of the patients initially aviruric began excreting virus during the follow-up period. In several of those initially excreting very low levels of virus the DNA became undetectable at follow-up, whereas in patients with a stronger reaction in the first urine, excretion seemed to increase. In several cases an increase in DNA detected seemed to correlate with a decreasing $CD_4$ cell count rather than with more stable high and very low cell counts. Similarly, in no patient was IgM detected at follow-up where it was not detected initially, and in four cases (1 BK, 3 JC) a low IgM of < 2 units in the first sample was undetectable 1 year later.

TABLE II. Correlation of BK IgM and JC IgM Antibody With DNA Detection in Urine

| | BK DNA detected | JC DNA detected | BK and JC DNA detected |
|---|---|---|---|
| BK IgM +ve (3) | 3 (100%) | 2 (66.7%) | 2 (66.7%) |
| BK IgM −ve (78) | 27 (34.6%) | 16 (20.5%) | 9 (11.5%) |
| JC IgM +ve (16) | 8 (50%) | 5 (31.3%) | 5 (31.3%) |
| JC IgM −ve (65) | 22 (33.8%) | 13 (20%) | 6 (9.2%) |

### Clinical Studies

Two patients developed PML during the study. In one patient, a 39-year-old female drug user, the symptoms and radiological findings were suggestive of PML. This patient had high stable JC HI titres (1,280–2,560) and low level JC IgM both initially and at follow-up, but only BK DNA was detected in the urine. A second patient, a 39-year-old homosexual, had PML diagnosed postmortem; in this case the JC HI titre was 320 with 1 unit of JC IgM; JC DNA was not detected. The patient was negative for BK and no follow-up specimens were obtained. In each of these two patients the $CD_4$ cell count was between 100 and 200/mm$^3$, and the $CD_4/CD_8$ ratio was, respectively, 0.15 and 1.06.

## DISCUSSION

In this study both BK DNA and JC DNA were detected frequently by PCR in the urine of patients immunocompromised by HIV infection. BK DNA was found in 37% of urine samples. This figure is similar to that reported previously in HIV-positive patients in the UK and Tanzania [Agostini et al., 1995; Jin et al., 1995], although lower rates of 20–24% were found in North America and Scandinavia [Markowitz et al., 1993; Sundsfjord et al., 1994] and recently 8% in Italy [Degener et al., 1997]. These results contrast with studies on groups of healthy individuals or nonimmunocompromised adult patients from three continents in which BK DNA was detected in < 8% of urine samples [Sundsfjord et al., 1994; Jin et al., 1995; Degener et al., 1997; Tsai et al., 1997], although in one study [Markowitz et al., 1993], 17.6% of urine samples from HIV-negative patients with risk factors for HIV infection were BK DNA positive.

The difference in prevalence of BK viruria between

TABLE III. Virological Studies on Patients Followed Up at 1 Year

| Patient no. | BKV HI titre I | BKV HI titre II | BKV IgM (units) I | BKV IgM (units) II | BKV VP1 I | BKV VP1 II | JCV HI titre I | JCV HI titre II | JCV IgM (units) I | JCV IgM (units) II | JCV VP1 I | JCV VP1 II |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial $CD_4$ cell count > 200/mm$^3$ | | | | | | | | | | | | |
| 1 | 160 | 160 | < 1 | < 1 | – | – | < 5 | < 5 | < 1 | < 1 | – | – |
| 2 | 80 | 80 | < 1 | < 1 | – | – | 80 | 40 | 1.2 | < 1 | – | – |
| 3 | 20 | 20 | < 1 | < 1 | – | – | 10 | 10 | < 1 | < 1 | – | – |
| 4 | 10 | 10 | < 1 | < 1 | – | – | 20 | 20 | 5.4 | 3.3 | – | – |
| 5 | 40 | 160 | < 1 | < 1 | – | – | 10 | 20 | 1.3 | < 1 | – | – |
| Initial $CD_4$ cell count 50–200/mm$^3$ | | | | | | | | | | | | |
| 6 | 80 | 160 | < 1 | < 1 | ++ | ++++ | 640 | 320 | 1.4 | 1.4 | ++ | +++ |
| 7 | 80 | 80 | < 1 | < 1 | – | – | 20 | 10 | < 1 | < 1 | – | – |
| 8 | 80 | 80 | < 1 | < 1 | ± | +++ | 20 | 20 | 1.4 | 1.4 | – | – |
| 9 | 10 | 10 | < 1 | < 1 | ± | N | 20 | 10 | < 1 | < 1 | – | – |
| 10 | 160 | 160 | < 1 | < 1 | – | – | 40 | < 10 | < 1 | < 1 | – | – |
| 11 | 2560 | 640 | 7.9 | 7.8 | ± | ++ | 20 | 20 | < 1 | < 1 | – | – |
| 12 | 10 | 10 | < 1 | < 1 | N | – | 5120 | 5120 | 79 | 46 | ++ | +++ |
| Initial $CD_4$ cell count < 50/mm$^3$ | | | | | | | | | | | | |
| 13 | 5 | 5 | < 1 | < 1 | ± | N | 5 | 5 | < 1 | < 1 | ± | – |
| 14 | 80 | 160 | 1.1 | < 1 | ± | ++++ | 160 | 160 | < 1 | < 1 | + | ++ |
| 15 | 40 | 40 | < 1 | < 1 | N | – | 10 | 10 | < 1 | < 1 | N | – |
| 16 | 160 | 160 | < 1 | < 1 | N | – | 10 | 10 | < 1 | < 1 | – | – |
| 17 | 80 | 80 | < 1 | < 1 | – | – | 10 | < 5 | 1.4 | < 1 | – | – |

I = initial sample; II = follow-up sample; ± to ++++ = estimated strength of amplimer; N = band visible only after nested PCR.

the HIV-infected groups studied may be accounted for in part by the overall level of immunosuppression within the groups. A significantly increased rate of BK viruria with lower $CD_4$ cell count (< 500/mm$^3$) has also been reported previously [Markowitz et al., 1993; Jin et al., 1995]. Increased levels of BK viruria were reported by Sundsfjord et al. [1994] and Degener et al. [1997] when comparing patients at CDC stages II, III, and IV, although the figures did not reach statistical significance.

In the present study, JC DNA was found in 22% of urine samples from HIV-infected patients, representing a rate of 35% of those who were JC seropositive. Neither $CD_4$ or $CD_8$ cell count or the $CD_4$/$CD_8$ ratio influenced JC viruria. These figures are in line with most other reports in which JC DNA was detected in the urine of between 16% and 32% of HIV-infected patients; excretion was independent of the $CD_4$ cell count [Markowitz et al., 1993; Sundsfjord et al., 1994; Agostini et al., 1995; Degener et al., 1997; Ferrante et al., 1997]. Furthermore, in other studies JC DNA has been reported in the urine of a similar percentage of healthy controls and non-immunocompromised patients [Sundsfjord et al., 1994; Agostini et al., 1996; Tsai et al., 1997], suggesting that the immunosuppression caused by HIV infection has little effect on the normal rate of JC viruria in the general population.

Several previous studies have found JC DNA excretion to be age related in non-immunocompromised patients and healthy volunteers, the prevalence rising to more than 50% in older age groups [Kitamura et al., 1990; Agostini et al., 1996]. In contrast, no such association was found in the present group of immunocompromised HIV-infected patients. Although BK DNA was detected in a higher proportion of patients older than 35 years of age than in those younger than 35 years in the present study, multivariable analysis showed that this finding was probably due to immunological status rather than to age. In immunocompetent subjects, the excretion rate of BK is too low for a clear association with age to be determined [Kitamura et al., 1990].

BK IgM antibody was detected in only 3% of the 94 patients in this study, but JC IgM was present in 17% of patients. These figures are similar to the rates reported previously of 3.7% and 15%, respectively, in blood donors in the U.K. [Brown et al., 1984; Knowles et al., 1992]. There was, however, no correlation between the detection of BK or JC IgM antibody and $CD_4$ cell count ($P \geq .9$). Previously, Flaegstad et al. [1988] reported a significantly lower prevalence and level of BK antibody in patients with AIDS than in those with ARC or healthy homosexuals, and were able to detect BK IgM in only one patient with ARC. Similarly, Sundsfjord et al. [1994] failed to detect BK IgM in any of 82 HIV-infected patients and found no significant correlation between the level of BK IgG or IgA antibody and the degree of immune suppression. Furthermore, although all three patients in our study with BK IgM had BK DNA in their urine, both BK IgM and JC IgM were poor predictors of viruria.

The clinical significance of both polyomavirus DNA detection in urine and IgM antibody is unknown. Viral DNA may be present in, or released from, latently infected cells shed from the urinary tract, and not represent active replication of virus. While infectious BK and JC have been isolated from the urine of patients with congenital immunodeficiency [Takemoto et al., 1974; Rosen et al., 1983] or those immunocompromised by treatment for tumours [Reese et al., 1975; Borgatti

et al., 1981], following renal [Gardner et al., 1984] and bone marrow transplantation [Apperley et al., 1987], or during pregnancy [Coleman et al., 1980], only molecular methods of detection have been applied to HIV-infected patients. However, DNA sequences from all regions of the genome (regulatory, early, late) of both BK and JC have been amplified by PCR in urine samples from HIV-positive patients indicating that the whole genome is present, although infectivity studies have not been reported.

The production of IgM antibody may indicate viral replication at a site other than the renal tract, and lymphocytes have been a focus of study. However, conflicting results have been reported. As with viruria, BK DNA was detected in a widely differing proportion of HIV-positive patients: 5% [Sundsfjord et al., 1994] and 51% [Degener et al., 1977]. Although Sundsfjord et al. [1994] failed to find JC DNA in the lymphocytes of any of 42 HIV-infected patients, in other studies 10–38% were positive [Tornatore et al., 1992; Dubois et al., 1996; Degener et al., 1997; Ferrante et al., 1997], but positivity did not correlate with $CD_4$ cell count. Tornatore et al. [1992] were unable to detect JC DNA in the lymphocytes of 30 immunocompetent patients with Parkinson's disease; however, Dörries et al. [1994] reported finding both BK and JC DNA in a high percentage of healthy adults and immunocompetent patients with Huntington's disease.

In our study, remarkably stable patterns for both BK DNA and JC DNA in urine and IgM antibody were observed in the specimens taken 1 year apart. Notably, no patient who was negative initially by PCR had begun to excrete virus 1 year later. These results were seen despite a marked fall in $CD_4$ cell count over the same period in some patients, and would indicate that factors leading to enhanced viral production, especially of BK, may occur soon after a critical $CD_4$ level is reached. In fact, it now seems that JC viruria is prevalent in non-immunocompromised adults in whom excretion can also be long-term [Kitamura et al., 1997], and is little affected by HIV infection.

The two cases of PML that developed in the present group of HIV-infected patients could not be predicted by either JC DNA detection or serology. In both patients, the $CD_4$ cell count was between 100 and 200 cells/mm$^3$: this is in agreement with previous reports in which most PML patients had a $CD_4$ count of < 200 cells/mm$^3$, with a mean of 85–89 cells/mm$^3$ [Karahalios et al., 1992; Fong et al., 1995; Ferrante et al., 1997]. Although both patients had low level JC IgM, JC DNA was not detected in urine from either. This finding was not surprising as only 55% of confirmed PML patients have detectable JC IgM [Knowles et al., 1995], and only 37–75% have JC DNA in the urine [Perrons et al., 1996; Ferrante et al., 1997].

The events that lead to the development of PML in a small percentage of HIV-infected (or other immunocompromised) patients are unknown. It has been postulated that virus may be carried to the brain in infected lymphocytes [Houff et al., 1988]. JC DNA was reported in peripheral blood lymphocytes of 89% of PML patients in contrast to 38% of other HIV-positive immunocompromised patients without PML [Tornatore et al., 1992]. Evidence of viral transcription, however, has been found only rarely [Dubois et al., 1997]. It has also been suggested that long-term enhanced JC virus replication in immunocompromised patients leads to genomic changes in the regulatory region producing variants that are able to invade the central nervous system. It was suggested early on that the structure of the regulatory region may be important in determining growth of JC virus in glial cells [Frisque et al., 1984]. Genome rearrangements, duplications, and deletions have been found in the regulatory region to the late side of the origin of replication in JC virus DNAs from the brains of PML patients, which could have evolved from an archetype sequence detected in urine of immunocompetent individuals [Yogo et al., 1990; Iida et al., 1993; Ault and Stoner, 1993]. However, several reports of the detection of low level JC DNA in the brain of patients without PML [Elsner and Dörries, 1992; Quinlivan et al., 1992; White et al., 1992] suggest that JC may invade the central nervous system at a much earlier stage of infection. Furthermore, once within the brain of HIV-infected patients, JC replication may be increased by the transactivating action of the HIV tat protein in dually infected patients [Tada et al., 1990].

BK and JC DNA are, therefore, detected frequently in the urine of HIV-infected patients with a $CD_4$ cell count of < 500 cells/mm$^3$, viruria being stable and long-term in a subset of patients. IgM detection correlates very poorly with viral DNA excretion, and neither JC serology or viruria can predict the onset of PML.

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